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Seed storage-mediated dormancy alleviation in Fabaceae from *campo rupestre*

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ABSTRACT

We studied the effects of seed storage on germination and dormancy alleviation in three species of Fabaceae endemic to *campo rupestre* in southeastern Brazil. Fresh seeds of *Collaea cipoensis*, *Mimosa maguirei* and *Mimosa foliolosa* were set to germinate and germination of seeds after four, five and 13 years of storage was tested. Seed viability was maintained for all species after the full storage period. Seed storage significantly increased germination percentage and decreased germination time for *C. cipoensis* and *M. foliolosa*, suggesting the alleviation of physical dormancy with storage. However, we did not find evidence of dormancy alleviation in *M. maguirei* since stored seeds showed a decrease in germination in comparison to that of fresh seeds. Our data indicate species-specific storage-mediated dormancy alleviation, which will have important implications for restoration of *campo rupestre*.

Keywords: *campo rupestre*, dormancy alleviation, physical dormancy, restoration ecology, seed longevity

Water-impermeable seed coats cause physical dormancy (PY) in seeds. PY occurs in 18 families of angiosperms and is a widespread trait across major biogeographic realms (Baskin and Baskin 2014). Physical dormancy is particularly important in Fabaceae (Baskin & Baskin 2000) where most species present water-impermeable seed coats irrespective of vegetation type. Seeds of many Fabaceae species form persistent soil seed banks and are expected to be long-lived since PY seeds are resistant to soil pathogens (Baskin *et al.* 2000).

Although the short-term effects of seed storage on seed viability are well-known (Rokich *et al.* 2000), the long-term storage effects on dormancy alleviation have not been tested in Neotropical legumes. Here, we artificially stored seeds of three Fabaceae species for different periods and tested for storage-mediated dormancy alleviation. Despite the fact that natural field conditions differ from those under controlled laboratory conditions, *ex situ* seed storage may provide useful data to advance our capacity to identify proper species for ecological restoration and germplasm banking.

Dry dehiscent pods ($n > 30$ individuals/species) were collected in *campo rupestre* vegetation at Serra do Cipó (19°17'S, 43°35'W), southeastern Brazil (Tab. 1). The *campo rupestre* is a species-rich altitudinal grassland highly threatened by cattle breeding, invasive species, uncontrolled fires, and particularly by quarries and opencast mining (Le Stradic *et al.* 2014). These communities establish on extremely nutrient-poor, quartzite-derived or ironstone soils, and harbour hundreds of endemic and/or threatened species (Giulietti *et al.* 1997). All study species are endemic from *campo rupestre* and have been recommended for ecological restoration (Negreiros *et al.* 2009; Le Stradic *et al.* 2014; Silveira *et al.* 2014). Seeds were collected from a single population for each species and were stored for different periods ranging from 48-144 months depending on seed availability (Tab. 1).

Fresh apparent viable seeds (seeds with no signs of predation by beetles) were sterilized with a 1% sodium hypochlorite solution for five minutes and then washed with tap water for 10 minutes. Seeds were placed in Petri dishes layered with double sheet of filter paper and moistened

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Table 1. Endemic species of Fabaceae collected at Serra do Cipó, southeastern Brazil.

Species	Growth-form	Microhabitat	Seed dispersal phenology	Storage time (months)
<i>Collaea cipoensis</i>	Shrub	Mesic	Dry season	48
<i>Mimosa maguirei</i>	Shrub	Xeric	Rainy season	60
<i>Mimosa foliolosa</i>	Sub-shrub	Xeric	Dry season	144

with distilled water whenever necessary. Apparent viable seeds were stored in 100mL opaque, closed glass pots at laboratory conditions in which temperature ranged between 13 and 29°C and moisture from 54 to 79%. To overcome PY, we mechanically scarified the seed coats of both fresh and stored seeds (Negreiros *et al.* 2009). We created four experimental treatments: 1) fresh/control, 2) fresh/scarified, 3) stored/control and; 4) stored/scarified. We set six replicates for each treatment, each one with 12-20 seeds, depending on seed availability. The Petri dishes were incubated under optimum conditions (25°C at a 12:12h light: dark cycle) and germination was scored upon radicle protrusion at daily intervals for 30 days. We calculated germinability (%), mean germination time (MGT) and germination synchrony (Ranal & Santana 2006).

To compare germination among all treatments, germinability, MGT and synchrony were analysed using permutation tests (Hothorn *et al.* 2008). Permutation tests are a type of statistical significance test in which the population distribution is obtained by calculating the sample statistics under every possible permutation of the observed data points, and such tests are appropriate for small sample sizes (Mestre *et al.* 2013). The p-values for the multiple comparison tests were recalculated with the BH adjustment (Benjamini & Hochberg 1995).

Average germinability of fresh and control seeds were < 15% for both *Mimosa* L. and 61% for *Collaea cipoensis* Fortunato. Viable seeds that did not imbibe were considered physically dormant. Species responses to storage were idiosyncratic. Stored seeds of *C. cipoensis* germinated to higher percentages compared to fresh control ones (maxT= 3.75, $p < 0.01$). The average germinability of *C. cipoensis* stored for 48 months was 94.4 (± 5.1 ; SE), with no significant difference from average germination of scarified seeds (Fig. 1A). Seeds of *M. foliolosa* Benth. stored for 144 months germinated more than control seeds but less than scarified ones (maxT= 3.45, $p < 0.01$; Fig. 1B). For *M. maguirei* Barneby, we found no difference between germinability of fresh and stored control seeds. Scarified seeds germinated >90% irrespective of scarification treatment while non-scarified seed germinated poorly (maxT= 3.45, $p < 0.01$; Fig. 1C).

MGT of stored and scarified seeds was nearly 3.5-fold less for *C. cipoensis* and 5-fold less for *M. foliolosa* (maxT= 3.40, $p < 0.001$; Fig. 1D, and maxT= 3.38, $p < 0.001$; Fig 1E. respectively). However, we found no differences in MGT for *M. maguirei*, irrespective of storage and scarification treatments (Fig. 1F; maxT = 2.11, $p = 0.134$). For *C. cipoensis*

and *M. foliolosa* neither storage nor scarification affected germination synchrony (maxT= 1.68, $p = 0.309$; Fig. 1G, and Fig. 1H, maxT= 1.39, $p = 0.52$, respectively). Conversely, germination synchrony decreased in scarified seeds of *M. maguirei* (maxT= 2.48, $p = 0.041$; Fig. 1I). Therefore, the storage behaviour of *M. foliolosa* was more similar to that of *C. cipoensis* than to *M. maguirei*, despite higher phylogenetic relatedness between both *Mimosa* species.

Our study showed that the effects of storage time on seed germination and dormancy alleviation in three legumes were species-specific. Physical dormancy was fully alleviated in *C. cipoensis*, partially alleviated in *M. foliolosa*, but was not alleviate in *M. maguirei* after *ex situ* seed storage. The relative increase in germination between stored and control seeds was also idiosyncratic, with a 5.45 fold increase in *M. foliolosa* and a 1.5 fold increase in *C. cipoensis*. Even short storage times (24-36 months) were sufficient to alleviate PY in a significant fraction of *M. foliolosa* seeds (Silveira *et al.* 2014). However, we recommend caution in interpreting our results since storage time was different for each species.

Physical dormancy in legumes is caused by the exotesta with a palisade epidermal layer of thick-walled Malpighian cells (Baskin *et al.* 2000). Here, seeds were stored under room temperatures for which daily and seasonal variations in air temperature and moisture were variable. Daily and seasonal temperature fluctuations are the most important factors breaking PY under natural conditions and may have accounted for changes in seed coat permeability in the study species (Baskin & Baskin 2000; Tieu & Egerton-Warburton 2000). Our results indicate that stored seeds had faster germination compared to fresh ones. If our data reflects what happens in nature, than species-specific responses to storage-mediated dormancy alleviation may have a direct impact in the community structure of seedlings emerging from soil seed banks. Nevertheless, studies carried out under field conditions are necessary to determine the role of *ex situ* topsoil storage as a technique for ecological restoration of *campo rupestre*.

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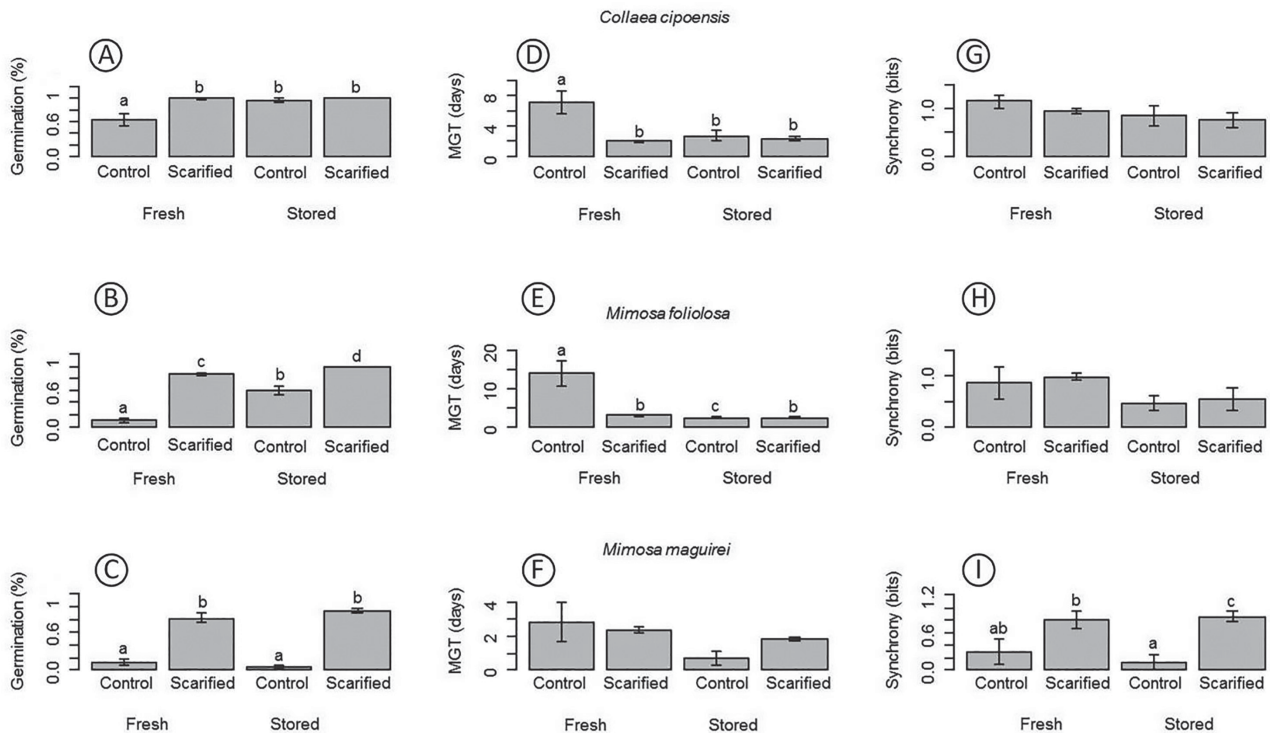


Figure 1. Mean (\pm SE) germination percentage (A, B, C), mean germination time (MGT) (D, E, F) and germination synchrony (G, H, I) of seeds of *Collaea cipoensis*, *Mimosa maguirei* and *Mimosa foliolosa* submitted to a combination of scarification and storage treatments. Different letters indicate significant differences following permutation tests and multiple comparison tests recalculated with the BH adjustment ($p < 0.05$). Low synchrony values indicate higher germination synchrony whereas high values denote low germination synchrony (Ranal & Santana 2006).

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